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THE FRIEDMAN TEST FOR PREGNANCY*

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THE purpose of this paper is to set forth in simple form the fundamental facts concerning the Aschheim-Zondek test for pregnancy, and its modification by Friedman. The use of the test in the diagnosis and treatment of hydatid mole and chorio-epithelioma will also be briefly reviewed. The Friedman test is so simple and practical in technique and interpretation that it is being used by an increasingly large number of workers, many of whom are not especially trained in laboratory procedure. We will consider the various limiting factors and sources of error with their relative importance to the dependability of the test.

DIFFICULTIES IN DIAGNOSIS OF PREGNANCY

The diagnosis of pregnancy has always been a source of great embarrassment to every practitioner of medicine. Errors are frequent and are not limited to the tyro in diagnosis. To attain great skill in pelvic diagnosis thousands of cases must be examined, but few men have access to the clinics affording this invaluable training. The differentiation between pelvic tumors and the pregnant uterus, the amenorrheas of endocrine origin, irregularities in menstruation, the menopause, and pregnancies occurring before the re-establishment of menstruation after delivery, are only a few of the problems that often leave the obstetrician or gynecologist in either the position of making a diagnosis with a sense of extreme apprehension, or refusing to commit himself, falling back on the watchful waiting policy which may be disastrous in certain instances to the health or reputation of the patient, and in certain others to the reputation of the physician. Patients do not like doubtful diagnoses.

It is no wonder, therefore, that the introduction of a laboratory test for pregnancy has always found an interested audience. The history of the various laboratory methods for the diagnosis of pregnancy and their failure as practical procedures is not within the scope of this paper. We are interested in the Aschheim-Zondek test and its modification by Friedman.

STUDIES BY ZONDEK, ASCHHEIM AND OTHERS

Zondek and Aschheim began a series of studies in 1925 which demonstrated the presence of the hormone of the anterior lobe of the pituitary body in the urine of pregnant women, and the fact that such urine when injected into sexually immature mice caused ovulation in the ovaries in about one hundred hours. This was one of the most important discoveries in recent endocrine investigation. Zondek [1] was able to state that "the anterior lobe of the pituitary and no other tissue of the body produces the hormone which sets in action the latent ovarian function, and thereby brings the infantile animal to sexual maturity." Evans [2] reported similar findings independently. Smith [3] reported that the anterior lobe hormone is a non-specific sex hormone; that is, it acts upon the male or female generative organs, bringing about development in either case. It was further demonstrated by Aschheim and Zondek that the ovarian hormone, not the pituitary, when injected into immature animals does not produce any change in the ovaries but apparently only in the uterus and vagina. The conclusion, therefore, is that the anterior lobe hormone brings the ovarian or follicular apparatus into action, fires off the follicular ripening and mobilizes the secondary ovarian hormone in the follicular cells. The ovarian or follicular hormone then acts in a specific way on the uterus and vagina.

The first attempt to develop a biologic test for pregnancy based on the above work was the demonstration of the ovarian hormone in small quantities of urine; but after considerable effort it was concluded that this method was not suitable because the hormone cannot be demonstrated in one to two cubic centimeters of urine earlier than the eighth to tenth week of pregnancy, and then not always with certainty. Moreover, the ovarian hormone may occasionally be excreted in large quantities in nonpregnant women, particularly those with functional disturbances such as the menopause, certain amenorrheas, hyperthyroidism, and myxedema.

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The blood of the pregnant woman shows a marked increase of both the pituitary and ovarian hormone. With the onset of pregnancy the rise of the ovarian hormone is gradual over a period of weeks. In contrast, the pituitary hormone rises rather acutely, a high level being reached a few days after conception. This remains high until the eighth month of pregnancy after which it gradually drops, reaching a normal level about the eighth day after delivery. This abrupt increase of the pituitary hormone, coupled with the fact that it reaches demonstrable amounts in the urine only in the presence of pregnancy, with two exceptions, hydatid mole and chorio-epithelioma, makes it the ideal hormone the determination of which makes possible an early and accurate diagnosis of pregnancy.

Let us summarize these important facts:

1. The hormone of the anterior lobe of the pituitary acts as the activating substance producing ovulation in the ovary.

2. The anterior lobe hormone reaches a high level in the blood, and is excreted in demonstrable quantities in the urine a few days following conception, the exact time as yet not having been determined.

3. The anterior lobe hormone is never excreted in the urine in demonstrable quantities in conditions other than pregnancy, hydatid mole, and chorio-epithelioma.

4. The ovarian hormone, not the pituitary, prepares the uterus for pregnancy, *i. e.,* produces hypertrophy and vascularization.

5. The ovarian hormone is excreted in the urine at a later period following conception, probably six to eight weeks.

6. It is present in the urine in demonstrable quantities in many other conditions, these conditions in several instances being the most important ones in which differential diagnosis of pregnancy occurs.

ASCHHEIM-ZONDEK TEST

The development of a practical method for determining the presence of the anterior lobe hormone was logically the next step, and the Aschheim-Zondek test was the result. A detailed account of the Aschheim-Zondek reaction will not be given. Suffice it to say that a positive Aschheim-Zondek test for pregnancy is based on the development of hemorrhagic follicles and corpora lutea in the ovaries of immature mice one hundred hours after the first of a series of six subcutaneous injections of urine.

The test has been used in almost every large clinic and by private laboratories all over the world. The statistics cover thousands of cases [4] and there is an almost universally recorded percentage of accuracy of from 98 to 99 per cent. The sole variations from these uniformly excellent results, in the literature reviewed, are the reports of Mazer and Hoffman,[5] White and Severance,[6] and Bland, First and Roeder,[21] reviewed later in this paper.

The Aschheim-Zondek test with mice, admirable though it is, has certain disadvantages. To use the test routinely it is necessary to be in a position to command a steady and dependable supply of immature mice of a definite age and weight. This means the maintenance of a large breeding colony of mice, numbering perhaps 5,000 to 10,000 animals, for, unless the demand for a given maturity of mouse be invariable, there is a constant loss from overmaturity. Mice are very susceptible to toxic substances in the urine, and the mortality is high. The interpretation of the results requires a magnifying glass for the examination of the ovaries and often requires serial sections. The test requires four to five days for completion although certain modifications involving the concentration of the urine have been proposed which may shorten the time factor considerably. The latter are still laboratory procedures not practical for the average worker.

FRIEDMAN'S MODIFICATION

To obviate these difficulties Friedman [7] proposed a modification of the Aschheim-Zondek test based on the fundamental facts that:

"1. The ovaries of an isolated immature female rabbit contain neither corpora lutea nor corpora hemorrhagica, as the rabbit does not ovulate spontaneously but only after coitus.

"2. The urine of a pregnant woman contains some substance or substances which simulate in their biologic effects the anterior hormone of the pituitary.

"3. The ovary of the rabbit quickly responds to the injection of these substances by the formation of corpora lutea and corpora hemorrhagica."

Friedman's technique as quoted from his article [7] is as follows: "The materials and equipment necessary for the performance of the proposed test are: (*a*) an ordinary bed-pan specimen of urine, (*b*) a five cubic centimeter syringe, and (*c*) an unmated mature female rabbit. The urine is injected intravenously thrice daily for two days in four cubic centimeter doses. Forty-eight hours after the first injection the rabbit is killed. If the ovaries contain either corpora lutea or large bulging corpora hemorrhagica, the reaction is positive and the patient who furnished the sample is presumably pregnant. If the ovaries contain neither corpora lutea nor corpora hemorrhagica but only clear unruptured follicles, regardless of their size, the reaction is negative."

LATER MODIFICATIONS

The original technique of Friedman has been modified in various ways: (1) Variations in the dose of urine. (2) Single as opposed to multiple injections. (3) Various attitudes toward the care of the urine sample with reference to the maintenance of its potency. (4) Suitable age of the rabbits and the care of the animals, particularly as to isolation, and selection of dependable stock. (5) Autopsy as opposed to exploratory laparotomy. (6) The time factor between the first injection of urine and the final examination of the ovaries. (7) The interpretation of the result.

DOSAGE OF URINE

Friedman and Lapham used four cubic centimeters of urine injected thrice daily for two days, a total of twenty-four cubic centimeters. They are the only workers reporting three cases of toxic urine which repeatedly killed the animals. This large and repeated intravenous dosage may have been responsible, for in no other series was such a large dosage used. Dosage by other workers varies from a five cubic centimeter single dose used by Wilson and Corner [8] to fifteen cubic centimeters used by MaGath and Randall [9] in one injection; Reinhart and Scott [10] at times also used fifteen cubic centimeters. The average dose seems to be seven to ten cubic centimeters in a single injection. The ten per cent error in a series by White and Severance [6] is partly attributed to the single dosage method. Friedman, in a discussion of this series, so places the blame and calls attention to the quantitative factor present.[6] "If one titrate the amount of this effective substance in the urine, he will find that in a seven months' pregnancy as little as one-sixteenth cubic centimeter of urine injected into a suitable rabbit in heat will give a positive response. If, however, one uses an adult animal, but an animal that is not in heat, many times that quantity will be necessary. In order to get an effective response of the urine of pregnancy with a minimal quantity, one must use a rabbit that is known to be in heat. One cannot tell from an external examination whether an animal is in heat." Therefore it is probably true that small amounts of urine if injected into one rabbit might give a positive, and into another, not in heat but apparently the same age and weight, might give a negative. For this reason Friedman uses large repeated doses. Reinhart and Scott [10] inject five cubic centimeters and do an exploratory laparotomy at twenty-four hours. If the test is negative they close the animal and inject a second dose of five cubic centimeters and kill the rabbit at forty-eight hours. The twenty-four-hour reading is rarely changed. A morning specimen of urine is desirable because of the concentration of the hormone. The greater dilution of specimens taken during the day might be the source of a false negative reaction.

THE STABILITY OF THE HORMONE

The stability of the hormone is a factor about which no specific data are recorded in the literature to our knowledge. It seems a simple question to clear up, but the cost of animals is rather high, and it is not surprising that the number necessary for this research has apparently not been available. Opinions regarding stability vary in the literature. Friedman says that if the material is handled properly (*i. e.*, kept on ice), the potency of the active sample will not be materially impaired at the end of six days.[7] MaGath and Randall[9] recommend that the urine be used within an hour of voiding, or if this is not practical it should be placed on ice, and urine older than five hours should not be used. Wilson and Corner,[8] who have done one of the most thorough and painstaking pieces of work in this field, store the urine on ice, and state positively that it remains active for months. We have no well-founded opinion on this subject. We make it a rule to place the urine on ice as soon as possible after it is voided, and inject the rabbit within three to four hours. One false negative in our series occurred when circumstances caused one of us to carry the sample for six hours on an extremely hot day. An additional factor in this instance, however, was an underweight, stunted fourteen weeks old rabbit, rendered so by a deficiency diet consisting solely of barley. It is apparent that no absolute statement on this important point can be made until specific research has established the facts. Dr. G. D. Maner of the Brem, Zeiler, Hammack and Maner Laboratories has done several hundred Friedman tests, and from his experience with specimens mailed to the laboratory, believes that there is little loss of potency over a period of some weeks.

SELECTION AND CARE OF THE ANIMALS

The rabbit does not as a rule copulate or go into heat until the age of five months during the summer, and six to eight months during the winter.[11] Therefore a rabbit of ten weeks is only half-grown, and one of twelve to fourteen weeks is still six weeks from maturity even in the summer months. The use of an immature animal, therefore, involves a certain period of time following the injection during which the ovary is being brought to maturity, following which, ovulation, the typical reaction of the test, occurs. Rabbits ten weeks old or less are definitely unsuitable for the Friedman test. Gladys Dodds,[12] in her first twenty cases, used rabbits less than twelve weeks old with 30 per cent failure. Her second group of thirty-three cases, using rabbits twelve to twenty weeks old, gave 100 per cent correct results. Schneider's [13] false negative occurred when for the first time six weeks old rabbits were used. Subsequent tests with twelve weeks old rabbits gave a correctly positive result. This fairly well establishes the fact that rabbits under twelve weeks old are not reliable and are apt to give false negatives. Rabbits at twelve weeks weigh four to five pounds.

Isolation Period.—An isolation period of at least three weeks for does who have been exposed to males is necessary to avoid false positives, for although pregnancy may not occur, the stimulation of attempted coitus may cause ovulation. At the end of three weeks, palpation will determine the presence or absence of pregnancy. Pseudo reactions may even occur from hopping of one doe by another. Therefore individual isolation of each doe is important.

METHOD OF EXAMINATION

Reinhart and Scott do an exploratory laparotomy at twenty-four hours, and if the test is positive they close the animal for future use; if negative, they close and reinject five cubic centimeters and reëxamine at forty-eight hours. They rarely find a positive at the second examination. Fried-

man kills his rabbits, as do the majority of workers; for although this method is less economical it does not involve the outlay of time and trouble necessary for laparotomy. Rabbits eight weeks old cost seventy-five cents to one dollar, and does twelve weeks old cost $1.75 to $2, from reliable uniform stock. A rabbit is ordinarily marketed at about ten weeks, and if saved longer represents a loss to the breeder.

THE TIME FACTOR

The period of time between the first injection of urine and examination of the ovaries varies in different laboratories from sixteen to forty-eight hours. Friedman used a forty-eight-hour period. MaGath and Randall use thirty hours, Schneider twenty-four to thirty hours, White and Severance forty-eight hours. The average and most universally used period is twenty-four to thirty hours. However, the errors in this test, with a few exceptions, are false negatives, and the several reported instances of negatives at twenty-four hours being positive at forty-eight hours indicate that perhaps the percentage of error could be still further reduced by always waiting forty-eight hours for the final reading. For example, Davis and Walker [14] opened a rabbit at twenty hours instead of the usual thirty hours and the reaction was negative. The rabbit died during the night, and on reëxamination in the morning was positive. Using mature does one investigator [15] opens the rabbit and sketches the ovary. The rabbit is then injected and reoperated upon eighteen to twenty-four hours later. In this way he can interpret actual changes in the ovary which have occurred as a result of the injection. He made hourly observations of the genitalia for a period of twenty-four hours. Hyperemia of the ovaries, tubes, and uterus occurred in three hours. A slight elevation of follicles occurred at the same time. This progressed until at the end of ten hours the picture of a positive test was present. Marked distention and coiling of the uterus appeared at six to seven hours.

Wilson and Corner [8] in using the sixteen to twenty-hour period with a low percentage of error always used fully matured rabbits, thus eliminating the time mentioned above during which the immature ovary is being brought to maturity by the hormone. Schneider [13] suggests that if there is a real necessity for rapid reading of the test, two rabbits may be injected, the first killed at twelve to sixteen hours, and, if positive, the second, of course, need not be sacrificed at the later period. This is a good idea. Urine concentration tests are being developed, and will probably soon be practical for use by the average worker, thus shortening the test safely.

INTERPRETATION OF THE TEST

In reading the test on immature virgin rabbits the specimens exhibited will show a striking difference between a negative and a positive. There is not available a description of a false positive caused by the sexual excitement created by females placed together. Occasionally small rosy spots appear in large clear follicles. These are suggestive, but not positive. Probably these should be retested in cases of very early pregnancy. When mature rabbits are used repeatedly the picture is more confusing. Undoubtedly the use of virgin rabbits is the safest and most practical for the beginner.

The Friedman test has a dependability of about 98 per cent in a majority of reported series, 100 per cent in a few instances, and in two series only 73 per cent and 90 per cent. What are the factors that make for error?

FALSE POSITIVES

A hydatid mole or chorio-epithelioma will give a positive reaction which quantitatively may be ten to fifty times as strong as that given by pregnancy. The reaction persists as long as any of the live tissue of either growth remains in contact with the maternal circulation. False positives from other sources are rare, and when carefully studied are usually accounted for by breaks in the technique of rabbit isolation or by mixed urine specimens. What may be called a false positive occurs in the case of incomplete abortion with retention of live placental tissue in contact with the maternal circulation. Bland, First, and Roeder [21] in their series reported 6.8 per cent false positives. "Especially," they say, "is this error likely to be encountered in women who are functionally sterile due to endocrine disturbances, or in women approaching the menopause. In these women a compensatory hypertrophy of the anterior hypophysis may produce an excessive quantity of hormone, which, finding its way into the urine, will render an incorrect positive." Pituitary hypertrophy following castration in the human being was noted by Tandler and Grosz, evidently an attempt to stimulate a poorly functioning ovary. Experimentally, Evans and Engle have shown that the hypophysis of gonadectomized animals possesses an activity five times greater than in the normal animal. This phase of the question of false positives is discussed in but one paper, a recent contribution. How important a factor of error it may be, apparently depends upon observations of future workers.

False Negatives.—False negatives are more frequent, and may be accounted for by the use of rabbits less than twelve weeks old or rabbits of uncertain or poor stock, insufficient dosage of urine, insufficient time between the first injection and final reading of the reaction. Missed abortions and ectopics may give negative reactions if the fetus is dead, and no live placental cells are in contact with the maternal circulation. It may be well to make an observation here regarding the collection of the specimen. Patients do queer things. We have all seen attempts at concealing pregnancy by patients who hope for operative procedures that may abort them. No report on a urine specimen should state that a certain patient is pregnant or not pregnant unless a responsible person has secured the specimen by catheterization.

SOME PERCENTAGE COMPARISONS

Considering the 10 per cent error in the series of White and Severance [6] it is well to note that they had a like variation from the experience of other reliable men in the use of the Aschheim-Zondek test. They had one false negative on a patient thirty-two days past her period with no explanation to offer. The other errors were two negatives on ectopic pregnancies. No comment was made on the condition of the fetus or placental tissue at operation. They had one negative incomplete abortion with no laboratory work on placental tissue secured at time of curettement, if curettement was done.

Brouha [16] did two hundred cases with 100 per cent results; Gladys Dodds [12] did twenty cases with six false negatives, an error of 30 per cent, using rabbits of one kilo and less than twelve weeks old. She followed this with a series of thirty-three cases, using rabbits twelve to twenty weeks old, with 100 per cent accuracy. She also changed the time factor from twenty-four to forty-eight hours on the second series.

Wilson and Corner [8] report:

Sixteen patients tested during the puerperium, all negative within twenty-four to seventy-two hours. In only two was the reaction positive longer than forty-eight hours, probably from retained placental tissue.

Eighteen patients in the first month of pregnancy from the menstrual history and development of the uterus, none more than thirteen days over her period, and three not more than eight days, all positive, and correctly so.

Thirty patients, four to eight weeks pregnant, all positive.

Three patients, ten to thirteen days past their periods, negative at the first examination, but four to seven days later became positive.

One patient (the earliest known) menstruated July 4, 1930. She was operated upon for a large myomatous uterus. No suggestion of pregnancy from history or physical examination. Hysterectomy was done July 24, 1930, and upon opening the uterus a three weeks' ovum was discovered. A specimen of urine was obtained eight hours after operation and gave a positive reaction.

Concerning twenty-five abortions, Wilson and Corner report:

Four, two to three months, threatened abortions were positive.

Nineteen incomplete abortions: Of these, ten were positive, nine negative. All giving a positive reaction showed placental tissue still attached to the uterine wall when curetted, and microscopic examination showed it to be living tissue. All negatives showed only decidua or dead inactive placental tissue.

Two missed abortions gave positive tests early, and negative later. No fetal cells.

Two cases of women at term, each with a macerated fetus dead one month or over. Both were positive. As is usual in these cases, the placentas were not macerated, and living placental tissue was present.

Six cases of ectopic pregnancy with three positive, being acute cases with living tissue, and three negative having had symptoms of longer duration and no living tissue.

This report of Wilson and Corner, a remarkably thorough study, is the type of work that will advance our knowledge of the reaction.

Schneider used fifty test cases. Twenty were positive and confirmed, and thirty were negative. Two negatives were later positive. Six weeks' rabbits were used for the first time on these two cases. Using twelve weeks' rabbits they were positive in twenty-four hours.

Dorn, Morse, and Sugarman,[17] in San Francisco, did 150 cases, and had three false negatives, using the twenty-four-hour technique. Two were subsequently positive, and one aborted before retesting. This gives them an error of 2.5 per cent. A forty-eight-hour technique might have reduced this to zero.

Davis and Walker [14] report one false negative at twenty-four hours, positive at forty-eight hours, and two false positives. These two tests were among the first done, and both rabbits were injected on the day they were brought to the laboratory, a bad break in technique.

In MaGath's and Randall's series of eighty-five cases, thirty-eight pregnancies were all positive, and forty-seven not pregnant were negative with one exception, another instance of a false positive. In this case there was a record of placing a young male in the same hutch with the doe used in the test.

Reinhart and Scott [10] had two failures in 150 cases. One false negative was operated upon, and two days after operation was positive. There was a very strong probability of a wrong source of the specimen used. The second failure was negative at twenty-four hours, but the clinical findings were so suspicious that a second rabbit was used, and forty-eight hours later gave a positive.

REPORT ON SERIES OF AUTHORS' CASES

We hesitate to report our series of thirty-five cases. The number is small, and insufficient time has elapsed for an accurate check-up.

REPORT OF CASE

The first case was interpreted without our ever having seen a positive, and we regret to admit that we called it positive. A few days later we realized our error, and repeated the test, which was again negative. The patient, a former nurse, felt sure she was pregnant, and would never have consented to interference at that time. Not having gained confidence in the test, and because the uterus proceeded to enlarge at a rate consistent with pregnancy, the patient was watched for two months. No fetal heart sounds developed. At operation a malignant tumor of the left ovary, superimposed over the uterus in the midline, was removed. Two false negatives in very early pregnancy occurred. The first, because of clinical symptoms, was rechecked two days later with the same single injection twenty-four-hour technique, and was positive. This was the instance in which the specimen was carried for six hours on a hot day, the rabbit used being one stunted in growth by a deficiency diet of cereal only. The second false negative was done three days after the patient had missed her period. It was repeated in two days, but was apparently repeated too soon, for the second reaction was also negative. The ovaries, however, in both instances showed clear large follicles. Friedman specifically calls these negative. Another worker recommends that the presence of these large follicles be an indication for repeating the test with an interval of five to seven days. During the succeeding ten days the patient was treated as an endocrine case, receiving antuitrin and theelin. Pelvic examination then showed uterine enlargement, and softening, and a third test using two injections of urine and a forty-eight-hour period gave a strongly positive result.

The patient at once left us for an abortionist, and was curetted. Of course no laboratory record is obtainable as to the presence or absence of pregnancy. As to the possibility of producing a false positive by the artificial introduction of the pituitary hormone, it is recorded in the literature that the transfusion of a patient with the blood of a pregnant donor will cause the recipient's urine to give a positive Friedman. Whether or not this patient was an instance of the artificial introduction of the hormone or was really pregnant we will never know. The case is reported to show the difficulty involved in obtaining reliable data.

HYDATID MOLE AND CHORIO-EPITHELIOMA

Only casual mention was made above of an extremely important function of the Friedman reaction, namely, its use in the diagnosis and prognosis of hydatidiform mole and chorio-epithelioma.

The urine of patients with hydatid mole or chorio-epithelioma gives a very strongly positive reaction. This excessive quantity of hormone was first noted by Zondek in April, 1929, and independently by Ehrhardt in November, 1929. In the case of a hydatid mole Ehrhardt found that one cubic centimeter of urine diluted 520 times gave a positive pregnancy reaction by the Aschheim-Zondek test, while a similar result was obtained in another case with one cubic centimeter of urine diluted 260 times. As the quantity of hormone in a liter of normal pregnancy urine is 10,000 mouse units, this means that in these two cases the hormone present was fifty-two and twenty-six times the normal, respectively. Therefore the quantitative determination of the anterior pituitary hormone in the urine may become of great importance in differentiating mole from pregnancy. This determination is probably best done by the Aschheim-Zondek test, using mice, as we have not yet determined a rabbit unit. Chorio-epithelioma gives a similar striking quantitative reaction.

After the expulsion of a hydatid mole the test may be used to check the subsequent clinical course. "The reaction may remain positive after hydatid mole as long as two months without evidence of chorio-epithelioma." [18] Theoretically, once the reaction becomes negative after expulsion of the mole it should not again become positive. However, such a case is reported by F. J. Browne in October, 1931,[19] and by Ehrhardt in 1930.[20] Repeated positive tests after the expulsion of the mole should, of course, be cause for keeping the patient under close observation for further evidence of developing chorio-epithelioma. Following the actual removal of a malignant chorio-epithelioma a continued positive reaction is evidence of metastasis, and reason for a grave prognosis.

CONCLUSIONS

The Friedman test has a high degree of accuracy of about 98 per cent which parallels that of the Aschheim-Zondek test, and exceeds that of other well-established laboratory tests such as the Wassermann reaction. This one to two per cent of error may be still further reduced by the application of a more standardized technique developed by longer experience with the test.

It is as yet uncertain as to exactly how soon after conception the reaction becomes positive, probably not under three weeks. Early pregnancies, if negative, should be checked a second time seven to ten days later.

No positive data are available concerning the duration of urine potency, but the hormone is now considered far more stable than earlier workers thought.

The test determines the presence of live placental tissue or tissue of placental origin in contact with the maternal circulation. It may, therefore, give false negatives in the presence of missed abortions, incomplete abortions, or ectopic pregnancies with dead fetal tissue. The reaction might be positive in the case of a macerated fetus as the placenta in these cases frequently contains live tissue.

The test is strongly positive in the presence of hydatid mole and chorio-epithelioma, and quantitative Aschheim-Zondek tests become an important aid in the diagnosis, treatment, and prognosis of these important conditions.

Attention has recently been called to the fact that primary ovarian failure or castration of the human being may cause a compensatory anterior lobe hypertrophy which may throw an excess of anterior lobe hormone into the circulation, thus accounting for a certain number of false positive reactions.

The technique that we suggest is the use of a fresh specimen of urine, with two injections of seven cubic centimeters each on successive days, using a carefully controlled rabbit not under twelve weeks of age, killing the animal at forty-eight hours. If greater speed is needed, two rabbits should be injected, as suggested by Schneider, one killed early, and, if negative, the second at forty-eight hours. Great care should be exercised to avoid injecting the wrong specimen of urine. This might be important legally. A catheterized specimen procured by a responsible individual is desirable.

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DISCUSSION

Frederic M. Loomis, M. D. (350 Twenty-ninth Street, Oakland).—Hardly a day passes when we are not called upon for the diagnosis of pregnancy too early for anyone to be certain. Doctor McNeile touched a tender spot when he said patients do not like doubtful diagnoses. I have discovered the same thing, especially since times have been so difficult. We have all probably had the same experience lately in seeing patients who would ordinarily welcome their babies go to the abortionists, feeling that they cannot possibly go ahead, and accepting this unhappy way out of their difficulty with fear and bitter resentment. When early diagnosis is so insistently urged, we can often suspect the reason.

We use the Friedman test constantly, done for us by Dr. Gertrude Moore, and have come to place practically complete reliance upon it. We have had no errors in many months. So far as I can remember we have had but two errors in all, a false negative early in the development of the test and a false positive after several injections of theelin. At the present time Doctor Moore is using carefully isolated rabbits, separated from other females also, from three and one-half to five months old. These are injected with four cubic centimeters of urine three times on both the first and second days at three-hour intervals, and the animal is killed at about forty-eight hours. The positives and negatives are easily identified in practically every instance, the tedious sectioning often required in mice being unnecessary.

I hope that the quantitative test for the early recognition of hydatid may soon become reasonably certain. We all have patients in whom we suspect hydatids and regret the wasted time when the diagnosis finally becomes clear; and at the same time we are sorely tempted at times to terminate suspected hydatids which finally prove to be normal pregnancies with a slight placental separation. We expect to follow our hydatids in the future with this test from time to time, though with a fairly long list of hydatids we have had no malignancy develop in the past fifteen years. Doctor Moore recently had a urine specimen produced two days after a hydatid was passed. This produced a clear positive, undiluted, and a clear negative when diluted ten times. In another patient the test was clearly positive, undiluted, after two weeks; this will be retested after another two weeks and if still positive it will be necessary to consider a strong possibility of malignancy.

Frank W. Lynch, M. D. (University of California Hospital, San Francisco).—The essayists have presented in a very clear and convincing manner their brief for the Friedman test for pregnancy. There is no doubt of the value of the method. Refinements in technique have made it an accurate means of diagnosis not only of early normal pregnancies, but for differentiating other confusing conditions from them. Moreover, the animal is a nice animal with which to work. No one in their right mind would prefer to handle rats or mice were rabbits available and suitable for the work. Moreover, as the essayist clearly shows, the availability of rabbits that are properly isolated makes it unnecessary to keep on hand the perfect swarm of rats or mice in the laboratory which one must do if he plans to have at all times animals of proper age for the Aschheim-Zondek test.

In my clinic we use for the pregnancy test rabbits, mice or rats, and in uncertain cases may use all three. While there are very few errors in diagnosis by this means, there are some even though the test is accurate in more than 99 per cent of cases. From our present standpoint of knowledge, we may be justified in believing that some of the failures accredited in the past to the fault of the method may more properly be laid to the use by technicians of animals of improper age. The difference of even two or three days in age in a rat or mouse may cause an error in obtaining a proper Aschheim-Zondek reaction if the animals are used at the earliest or latest accepted age period for the test. Concentration methods of preparing the urinary specimen speed up the interval of time before a test can be made.

Equally important is the selection of rabbits of proper age for the test. Wilson and Corner in their most excellent study obtained their astonishing results with the use of mature rabbits that were properly isolated. The ovaries of two or two and a half months old rabbits may be too immature to respond to the stimulation of the hormones contained in the urine of pregnant women. In this connection it is of interest that Dorn and Sugarman, when working out their test for the determination of the sex in the unborn, found that a rabbit whose testicles were still in the abdominal cavity was too undeveloped to respond to the hormones in the urine of a woman carrying an unborn female child, whereas those with testicles in the process of descent and still within the inguinal canal did so respond.

The ages when rabbits go into heat, *i. e.*, give evidence of maturity, varies in different parts of the country under the influence of their breed, size, food, and climatic conditions. They are said to mature at four and a half months in the summer and in five months in the winter in the East, yet it is a fact that they mature at least two weeks earlier in this section of California. We also find that rabbits bred in the San Joaquin valley go into heat earlier than those bred in the coast counties near San Francisco.

The essayists stress the great value of the Friedman and Aschheim-Zondek test as a means of differential diagnosis when trophoblastic tissue is supposed to be present. Our observations confirm this statement.

We have used the test in several cases of hydatidiform mole, beginning shortly after Aschheim, in March, 1930, calling attention to the fact that the urine of patients with such complications gave a very much stronger Aschheim-Zondek reaction than did that from women with normal pregnancies. We have had several cases which corroborated this statement. Yet the clinician should remember that although the test usually becomes negative a comparatively short time after the extrusion of the mole, it may remain positive as long as two months thereafter without the presence of chorio-epithelioma. In one of our cases the reaction was negative nine days after the mole was removed from the uterus. Eberhardt, however, found it was positive with undiluted urine for thirty-six days in one of his patients, and in forty-two days in another. In November, 1930, the test proved consoling to us when treating a patient with hydatidiform mole who bled considerably for one month after removal of the tumor. Immediately after the operation the test was positive for mice with one-eighth of one cubic centimeters of urine in three days (Aschheim-Zondek technique) as opposed to the normal pregnancy reaction then obtained with one cubic centimeters of urine in four days. A month later we found two large ovarian cysts (lutein) in the abdomen that were not present at the time when we removed the tumor. Since the patient was bleeding, we curetted without finding syncytial elements, yet because she was forty years of age and had had several children, to be on the safe side we inserted a small dose of radium to spray completely the uterine cavity. Ten days later the Aschheim-Zondek test was negative both for mice and with the concentrated method for rats. In the light of present knowledge, we would not use radium in treating such a patient today.

Quite recently there has been in one of the University Medical School Hospital services a most perplexing case. In March the patient was delivered of a mole. Two weeks later the Friedman test for pregnancy was positive, using ten cubic centimeters of undiluted urine, and then on May 2, May 16, and on August 27 it was still positive, even when using ten cubic centimeters of urine diluted 1:50 and 1:100 times. This long continuation of a very strong pregnancy reaction, together with some bleeding, seemed to indicate the presence of a chorio-epithelioma. The woman consequently was curetted without such findings. Feeling that this reaction, continued for more than four months, demanded more careful investigation, the abdomen was opened without finding evidence of tumor. Two weeks, and three weeks thereafter the reaction was negative, using ten cubic centimeters of 1:100 solution of urine. While there is always the possibility that the curette may fail to give the diagnosis, even though the tumor may be present, as in the case reported by Browne, the negative Aschheim-Zondek reaction indicates that at present there is neither trophoblastic tissue nor tumor. At any rate, the woman seems well and is symptom-free. This case will be reported later in detail.

Within the last few weeks the Aschheim-Zondek reaction has aided us in diagnosing an ectopic pregnancy. This patient's July period came a few days early; then she bled normally from August 21 to 26, bleeding returning August 29 to September 2, without pain or distress. On September 6 she had several attacks of cramping pain which continued off and on for a couple of days but which was relieved by taking aspirin. In all, she had but four such crampy attacks which were later replaced by pain running down her thigh. She applied to my service on September 12, having a small right tubal mass. At this time an Aschheim-Zondek test was positive. We tentatively diagnosed an early tubal abortion which did not seem confirmed by the blood picture when she entered the hospital on Septmber 18, when the red blood count was normal, the white blood cells were 7720, with 67 per cent of polymorphic leukocytes. The blood sedimentation time was three hours and twenty-minutes. Yet operation on the following day proved the tentative diagnosis was correct. The Aschheim-Zondek test was positive on the day of operation, and seven days later, but was negative two weeks after operation. While the specimen presented grossly only as a tubal abortion, microscopic study disclosed several small areas of chorionic villi still firmly attached to the walls of the tube in a few places.

The use of this diagnostic aid suggests a wider field for application. The reaction, supposedly due to living chorionic villi, may be due only to the fetal epithelium as is suggested by Eberhardt's case of chorio-epithelioma in which only remote metastases were found without the uterine tumor. It well may be that the varying strengths of the test may prove useful in the event patients with normal pregnancy may have unduly large amounts of syncytial cells in the lung and liver which cause such symptoms as hyperemesis or other toxemias of pregnancy which at present remain as unsolved problems.

Doctor McNeile's paper is timely and presents a critical review of the literature which should be of interest to any advanced student in obstetrics.

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H. A. Stephenson, M.D. (490 Post Street, San Francisco).—Doctors McNeile and Reynolds have surely given us a complete résumé of the literature on this subject. Since it is becoming increasingly necessary to make a positive and early diagnosis of pregnancy we are coming more and more to rely on the Friedman test. We agree with Doctor McNeile that it would be much wiser to have the urine collected from the patient by a nurse so that we may be absolutely sure that the specimen is the proper one. We have not taken this precaution in the past, but shall do so in the future. We have had no experience with the technique of the test as we have depended upon reliable laboratories. Done in this way the test has been in our practice 100 per cent successful.

WASSERMANN-FAST SYPHILIS*

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Discussion *by Samuel Ayres, Jr., M. D., Los Angeles; Donald A. Charnock, M. D., Los Angeles; Hermann Schussler, Jr., M. D., San Francisco.*

THE term "Wassermann-fast" is given to a case of syphilis that still shows a positive Wassermann reaction after having been treated over a period of three or more years by ordinary chemotherapeutic methods. This includes any combination of the arsphenamins, bismuth, mercury, and the iodids. For the purpose of this paper we will consider only those cases in whom all clinical manifestations of syphilis have been arrested, and we will also exclude cases of paresis.

SIGNIFICANCE OF THE WASSERMANN-FAST REACTION

Following our best interpretation of the Wassermann reaction we find it is supposed to be positive when certain enzymes are circulating in the blood. These enzymes result from the antigenic action of certain foreign lipoid-protein mixtures or compounds, such as the bodies of dead spirochaetes. The spirochaete multiples by simple fission and if its environmental conditions are favorable the spirochaete does not ever need to die. In conditions where no spirochaetes are being

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